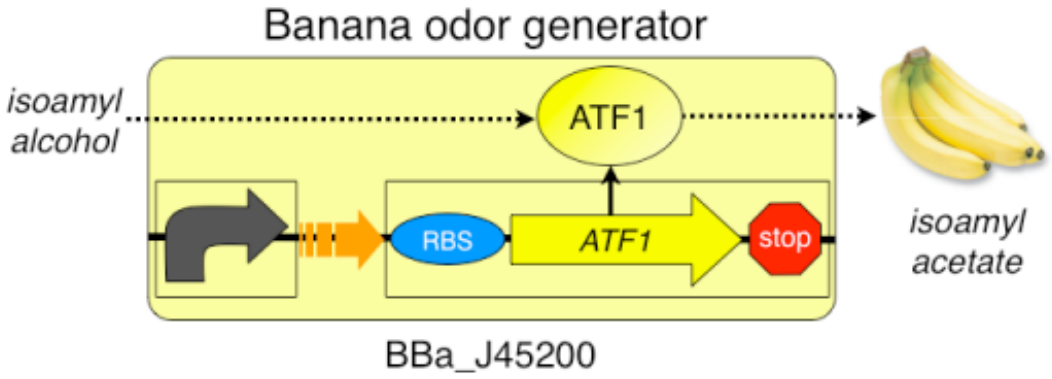
LAB 1: Eau that smell

- Compare 2 competing designs to optimize system performance



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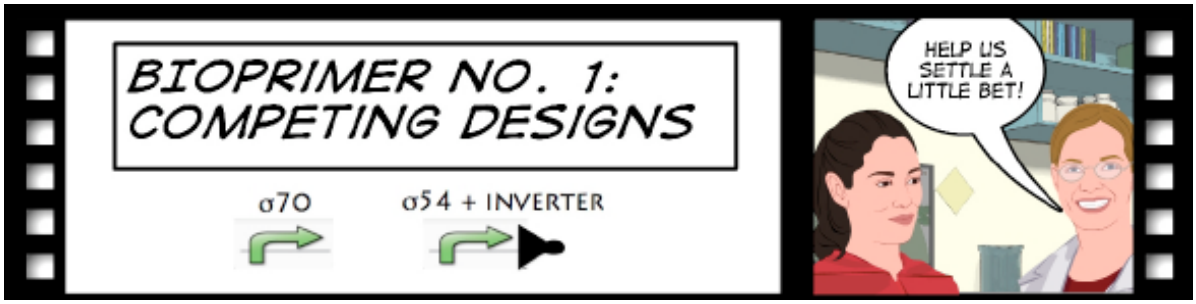
Objectives

By the conclusion of this laboratory investigation, the student will be able to:

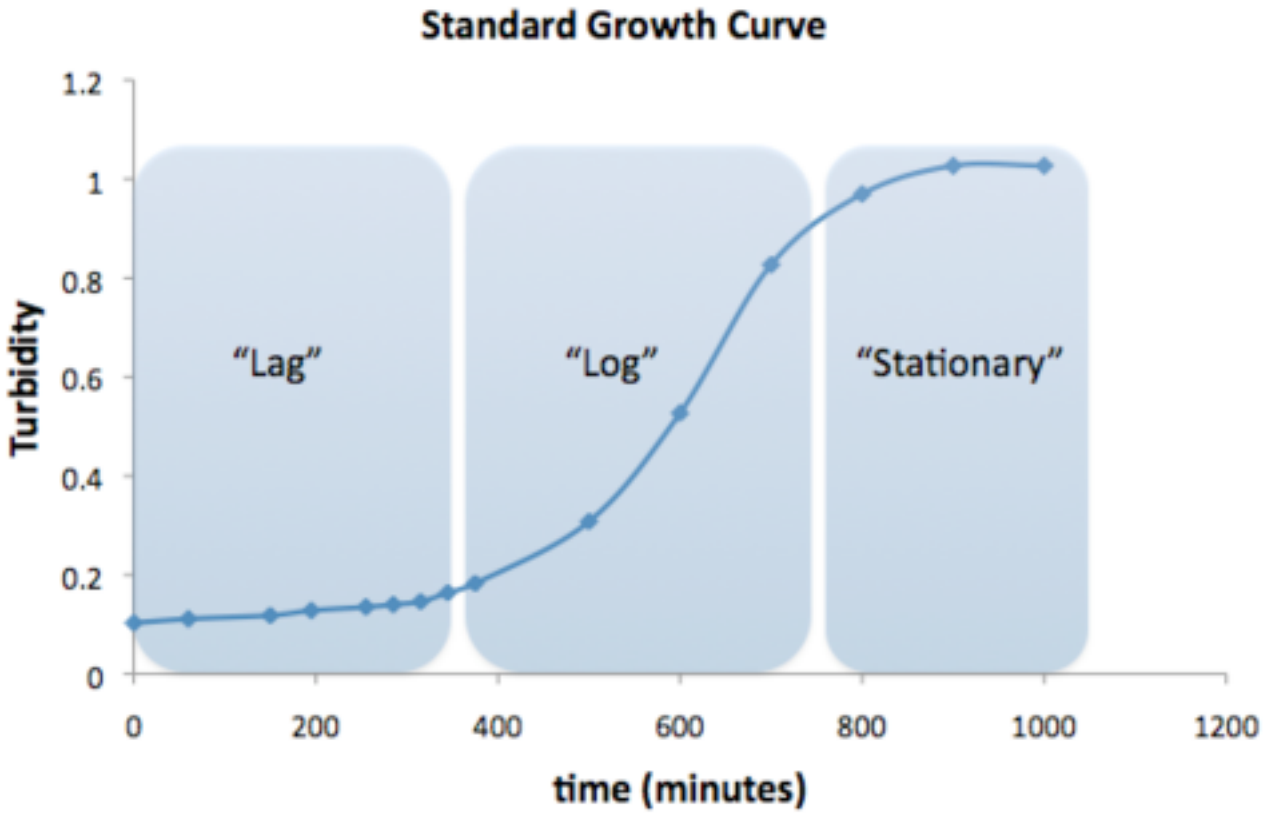
- Explain how synthetic biology as an engineering discipline differs from genetic engineering.
- Explain the population growth curve of bacteria.
- Culture bacteria using proper microbiology methods.
- Measure the growth of a bacterial population.
- Define and properly use synthetic biology terms: Part, Device, Inverter.
- Define and properly use molecular genetics terms: Promoter, ribosome binding site ("RBS"), open reading frame ("ORF"), Terminator, Plasmid.

Introduction



For the 2006 iGEMcompetition, MIT students designed Eau d'Coli, *E. coli* that smell like bananas when their population is in the stationary phase. They did this by inserting device that contains a stationary phase sensitive promoter coupled to a banana smell device, a device that contains a ribosome binding site (RBS), an open reading frame (ORF) that codes for the ATF1 enzyme and terminator sequences. The ATF1 enzyme converts isoamyl alcohol to isoamyl acetate, the molecule that gives bananas their characteristic smell.

It has been suggested that a device that generates the banana smell during the bacteria's log (or exponential) phase of population growth will be helpful. There are two ways to accomplish this. Both methods will continue to use the banana smell device but alter the function of the promoter. One method involves coupling the banana smell device to a new part, a log phase promoter. The other method involves using the same promoter but adding an inverter. Synthetic biologists have constructed these devices for us and transformed bacteria with them.



We have been sent four strains of *E. coli*. Each contains a different device:
Sample 1-1. The original Eau d'Coli device
Sample 1-2. The original Eau d'Coli device but with an inverter added between the promoter and the RBS.
Sample 1-3. The banana smell generator coupled to the log phase promoter
Sample 1-4. A strain of E. coli that has no smell generating devices.
Our task will be to grow these bacterial populations and test for the banana smell as the population moves through the log phase and into the stationary phase. We will determine the population growth by using a spectrophotometer or the McFarland Turbidity Standards to measure the density of the bacteria in liquid culture. As the population increases we can assess the increasing banana smell, comparing the smell to dilutions of banana extract.

Procedure

Day 1

We will be receiving our bacteria with the plasmid already inserted. This culture will come in the form of a "stab" or "slant", a test tube with a small amount of bacteria on a slanted media. To continue the experiment we will have to further culture the bacteria by streaking out the stabs onto LB+amp plates. The plates will be incubated 37° overnight.

1. Using a sterile toothpick or inoculating loop, gather a small amount of bacteria from the stab and transfer it to a petri dish containing Luria Broth (LB) agar plus ampicillin medium.
2. Repeat with the remaining stab samples, streaking out each onto a different petri dish.
3. Place these cultures in a 37°C incubator overnight.

This video illustrates the technique used for this transfer.

Day 2:

1. Using a sterile inoculating loop, transfer a bacterial colony from one of the petri dishes to a large sterile culture tube containing 5 ml of Luria Broth and 5 µl of ampicillin.
2. Repeat for each strain you will inoculate.
3. Place the culture tubes in the roller wheel in the incubator at 37°C overnight. Be sure to balance the tubes across from each other to minimize stress on the roller wheel.

This video illustrates the general technique for setting up overnight liquid cultures, though you'll be transferring cells from the petri dish to the Luria Broth.

Day 3, 4, 5:

Procedure if using a spectrophotometer

1. Prepare a stock growth solution with
    - 300 ml Luria broth
    - 300 µl Ampicillin
    - 250 µl isoamyl alcohol
2. Mix this stock growth solution, by swirling the bottle or vortexing gently.
3. Set aside 2 ml of this mixture for each student group into a small sterile culture tube. This aliquot will serve as the blank for the spectrophotometer.
4. Move 50 ml of the broth solution to 100ml sterile erlenmeyer flask and add 2ml of bacteria from one of the overnight cultures, e.g. strain 1-1.
5. Repeat the addition of 2ml of bacteria to 50 ml of broth in the erlenmeyer flasks for each of the overnight cultures.
6. Cover the flasks with foil and start them gently stirring on the stir plates.
7. Remove 2 ml from each sample to read the starting density of each. If you are testing all 4 samples you should now have 5 small test tubes (4 with bacterial dilutions and one blank).
8. Prepare the spectrophotometer by setting it to OD600.
9. Note the time and take an "initial" density reading for the bacterial samples. Please note that your teacher may have carried out the preceding steps in advance of the lab. If that is the case, the teacher will tell you how much time has elapsed. That time will be your T_0.
10. Add a stir bar to each culture flask and place onto stir plates. Stir slowly. Cover the flasks with foil.
11. After 20 minutes, remove 1-2 ml from each sample and place in a cuvette. Note: the volume you use here will depend on the size of the cuvette appropriate for your spectrophotometer. Please follow the teacher's instructions.
12. Read the blank and adjust the % Absorbance to zero.
13. Read the sample tubes and record the % Absorbance.
14. Sniff the flask for any evidence of a banana smell, comparing the smell with the banana extract standards. Be sure to shake the standards and the cultures before sniffing. Record your data.
15. At 20 minute intervals repeat steps 11-14.
16. Between time points, you can calculate the bacterial population: 1 OD600 unit = 1 x 10^9 bacteria.

Procedure, if no spectrophotometer is available

The turbidity of the bacterial populations can be estimated using the McFarland Turbidity Scale. This method uses suspensions of a 1% $BaCl_2$ in 1% H_2SO_4 that are visually similar to suspensions of various populations of *E. coli*.

1. Following your teacher's instructions, obtain small clear test tubes containing the turbidity standards. The tubes should contain enough standard in each to fill the tube to a height of about 1 inch (2.5 cm) from the bottom. Make sure each tube is properly labeled with its turbidity standard number. If you are filling the tubes from stock bottles of the standards, use small tubes and place enough standard in each to fill the tube to a height of about 1 inch (2.5 cm) from the bottom.
2. Place the samples in a test tube rack that allows you to view them from the side. Use small tubes and place enough standard in each to fill the tube to a height of about 1 inch (2.5 cm) from the bottom.
3. On a blank index card or paper use a marker to draw two thick black lines. These lines should be within the height of the standards.
4. Place the card with the lines behind the standards.
5. To compare your bacterial cultures to the standards, you will need to place the bacterial sample in a test tube of the same size and equal volume as the standards. be sure to label these sample tubes.
6. Place the sample tube next to the standard tubes. You should move the sample to compare it to the standard tubes with the most similar turbidity. You can make this assessment more precise by looking for a standard that most similarly obscures the black lines on the background card.
7. Use the table below to determine the comparable OD 600.
8. 1 OD 600 unit equals approximately 1 x 10^9 cells.

Turbidity Scale	OD 600	1% $BaCl_2$/ 1% H_2SO_4 (mL)
0	0	0.0/10
1	0.1	0.05/9.95
2	0.2	0.1/9.9
3	0.4	0.2/9.8
4	0.5	0.3/9.7
5	0.65	0.4/9.6
6	0.85	0.5/9.5
7	1.0	0.6/9.4

Data Table

In your lab notebook, you will need to construct a data table as shown below for each of the samples.

SAMPLE_____

Time (minutes from T_0	OD 600	cells/ml*	Banana smell (0-6)
T_0 (initial)			
T_0 +			
T_0 +			
T_0 +			
T_0 +			
T_0 +			
T_0 +			
T_0 +			

- 1 OD600 unit ~ 1 x 10^9 bacterial cells/ml

Lab Report

As you write, be sure to define and properly use all highlighted terms throughout the introduction and other parts of the lab.

I. Introduction

- Provide a brief introduction describing the field of synthetic biology.
- Briefly describe the purpose of the lab. What are we trying to do here?
- Explain how the banana smell generator functions.
- Why are we using optical density to measure the population?
- Explain each phase of the bacterial population growth curve.
- Presume that a reader of your lab report has not read the assignment.

II. Methods

- You do not have to rewrite the procedure.
- Explain why you did each step of the protocol.

III. Results

- Present the data tables in clear format.
- Draw population growth curves of the class mean data for each sample. Indicate on each curve when you could smell bananas.

IV. Discussion

- Describe the results: Were we able to measure the population growth? Were we able to smell bananas? Did each device produce the same results? Did the genetic systems affect the growth curve of the bacteria? Explain your answers.
- Analyze the data: Be sure to discuss how each part of the experiment adds to your conclusion.
- Discuss errors and other reasons for data variability.
- How confident are you in the results? Are you equally confident in both the growth data and the smell data? Explain.
- Is using smell to measure the banana smell valid? Why or why not?
- What did methods did you use to try to increase your confidence in the results?
- How might we try to change this system so that we can quantify the banana smell? Would we be better off using a different kind of signal? If so, what would you suggest?
- If you could construct a different genetic system, what might you construct? What would you need to do?

V. Citations and references

- Be sure these are of good quality.
- Embed citations.
- Follow proper reference format.